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TITLE: NSAIDS and the Osteogenic Response to Mechanical Stress in Premenopausal Women

PRINCIPAL INVESTIGATOR: Wendy M. Kohrt, Ph.D.
Robert S. Schwartz, M.D.

CONTRACTING ORGANIZATION: University of Colorado Health Sciences Center
Aurora, CO 80045-0508

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Robert S. Schwartz, M.D.

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14. ABSTRACT

This is a study of the effects of ibuprofen, a non-steroidal anti-inflammatory drug (NSAID), on the osteogenic response to 9 months of exercise training in healthy, premenopausal women, aged 21 to 40 years (N=102). The hypotheses are: H1a: taking short-acting NSAIDS before exercise will diminish increases in bone mineral density (BMD) in response to exercise training H1b: taking short-acting NSAIDS after exercise will not diminish the increases in BMD in response to exercise training Participants take either ibuprofen (400mg) or placebo capsules before and after each exercise session. Women are randomized to three treatment arms: 1) NSAID before exercise, placebo after exercise (NSAID/placebo; n=34); 2) placebo before exercise, NSAID after exercise (placebo/NSAID; n=34); and 3) placebo before exercise, placebo after exercise (placebo/placebo; n=34). One hundred thirteen women completed baseline testing and were randomized to treatment. Final follow-up testing was completed approximately 7 months ago and most sample analysis has been completed. Re-analysis of some samples and review of the database continues for quality assurance. Manuscript preparation is underway. These studies could lead to the development of new strategies to reduce the incidence of, and treatment for, stress fractures that occur in response to vigorous physical activity.

15. SUBJECT TERMS
Exercise, stress fracture, ibuprofen, prostaglandins, bone mineral density, estrogen

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INTRODUCTION:

The primary aim of this randomized, double-blinded, placebo-controlled trial is to determine the effects of NSAID (ibuprofen) use on the osteogenic response to 9 months of exercise training in 102 women. The scientific rationale for this study centers on the knowledge that the osteogenic response to mechanical stress is a prostaglandin (PG)-dependent process and that NSAIDs inhibit PG synthesis. There is evidence that regular NSAID use inhibits the normal bone formation response to mechanical loading, increases risk of fracture, and impairs bone healing. The approved statement of work for this project included 4 years for recruiting, testing, and training subjects and completing sample assays, data analysis, and manuscripts. Completion of the trial took longer than expected, but a no-cost extension was requested to facilitate publication of results.

BODY:

The objectives for this year were to complete all follow-up tests, procedures, and biochemical assays, perform data analyses, and prepare manuscripts. All follow-up visits have been completed and most sample analyses have been completed (i.e., re-analysis continues for samples for which level of agreement of duplicate measurements was not within the desirable range). Quality assurance evaluations of the data are being performed but have not been completed. Preliminary statistical analyses are underway, but final analyses and publication of results will not be performed until after the database is finalized. A no-cost extension of the award period was requested so that work on the project can continue.

Figure 1 illustrates the flow of volunteers through the study. The attrition rate of 34% was higher than the projected rate of 25%. More women were randomized to treatment (n=113) than originally proposed (n=102) to help offset the attrition. In the original proposal, it was estimated that 19 finishers per group would be required to achieve β<0.020 (i.e., power of 80%). It was also proposed that primary analyses would be based on compliance, rather than intent-to-treat, because the study was not a clinical trial. Of the 73 women who completed the intervention, the number of participants in each group that were compliant to treatment has not yet been finalized. Based on the power analyses in the grant proposal, we need 19 compliant finishers per group (N=57) to achieve 80% power. The racial and ethnic characteristics of the study participants are similar to those that were projected, which reflect the demographics of the Denver metropolitan area (Table 1).

Changes in body composition and bone mineral density (BMD) in response to exercise training are depicted in Figures 2 and 3, respectively. Two outliers were omitted from the data set from which these figures were generated because their changes in BMD were more than 4 SDs different from the mean change (1 in the placebo/ibuprofen group, 1 in the placebo/placebo group). Changes in fat mass were similar among the groups. Although we did not hypothesize that taking ibuprofen before exercise would affect bone *and* muscle metabolism, a study that was published after this project was initiated provided evidence that ibuprofen impairs the increase in muscle protein synthesis in response to exercise.[1] Thus, it is intriguing that the smallest increase in fat-free mass occurred in the group that took ibuprofen before exercise (Figure 2). Consistent with our hypothesis, changes in BMD in the group that took ibuprofen after exercise were larger than in the group that took ibuprofen before exercise. It should be noted that statistical analyses of these data have not yet been performed because censorship for noncompliance to the intervention has not yet been finalized. We expected that the largest increases in BMD would occur in the placebo/placebo group, but preliminary examination of the data does not support this. We will evaluate whether the blunted response in this group

(compared with what was expected) can be explained by poor compliance to exercise or to non-study-related use of NSAIDs.

Preliminary results from the bone marker (bone resorption – CTX; bone formation – BAP), sex hormone (estradiol, testosterone, progesterone, sex hormone binding globulin), and gonadotropin (luteinizing hormone, follicle stimulating hormone) assays are depicted in Figures 4-7. No statistical analyses have been conducted because datasets have not been finalized.

Table 4 presents a summary of dietary records and measurements of maximal oxygen consumption (VO_2max). These data indicate that energy and nutrient intake remained relatively constant over the period of study and that the endurance component of the exercise program was sufficiently intense to generate an increase in VO_2max. There is one cautionary note regarding the dietary data. The diet records were analyzed by the Bionutrition Core of the UCDHSC General Clinical Research Center (GCRC). In the past year, we learned that the Core staff did not use the same software application to analyze all of the food records for this protocol. This is expected to introduce variability to the results because applications use different databases of food products. The diet variables are not key outcomes for the study, so this is not expected to have a major impact on out ability to interpret or publish the findings.

KEY RESEARCH ACCOMPLISHMENTS:

The key accomplishments to date have been the completion of enrollment and testing subjects. Quality control evaluations to finalize the database has taken longer than anticipated but good progress has been made. It is expected that formal data analyses and preparation of manuscripts will commence within the next 1-2 months.

REPORTABLE OUTCOMES:

The investigators remained blinded to treatment status until all follow-up tests had been completed. No outcomes could be reported.

CONCLUSIONS:

Conclusions cannot yet be drawn because final data analyses have not yet been conducted. This remains a completely novel area of investigation in humans. We are not aware of any intervention studies that have evaluated whether non-steroidal anti-inflammatory drugs (NSAID) impair the osteogenic response to mechanical loading. As discussed above, one study published after the current study was initiated found that the fractional muscle protein response to a single bout of resistance exercise was blunted by ibuprofen.[1] The same investigators also reported that the increase in muscle prostaglandin levels in response to a single bout of exercise was blunted by ibuprofen.[2] Thus, data analyses for the current study will evaluate whether both bone and muscle (i.e., fat-free mass) adaptations to exercise are blunted when NSAIDs are used prior to exercising. The importance of the timing of NSAID administration relative to mechanical loading, which was identified in the original grant application,[3] has been reinforced by another group of investigators.[4]

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1. Trappe TA, White F, Lambert CP et al. Effect of ibuprofen and acetaminophen on postexercise muscle protein synthesis. *Am J Physiol* 2002; 282:E551-E556.

2. Trappe TA, Fluckey JD, White F et al. Skeletal muscle PGF(2)(alpha) and PGE(2) in response to eccentric resistance exercise: influence of ibuprofen acetaminophen. *J Clin Endocrinol Metab* 2001; 86:5067-5070.
3. Chow JW, Chambers TJ. Indomethacin has distinct early and late actions on bone formation induced by mechanical stimulation. *Am J Physiol* 1994; 267:E287-E292.
4. Li J, Burr DB, Turner CH. Suppression of prostaglandin synthesis with NS-398 has different effects on endocortical and periosteal bone formation induced by mechanical loading. *Calcif Tissue Int* 2002; 70:320-329.

APPENDICES:

Figure 1. Study participant flow chart

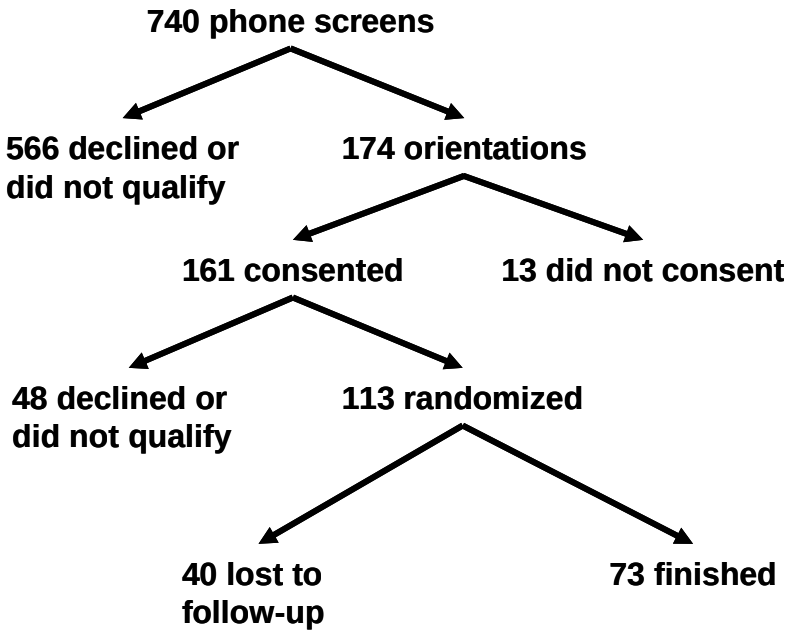

Table 1. Projected and actual enrollment by ethnicity and race

Race/Ethnic Category	Actual Enrollment	% Total	Projected Enrollment	% Total
RACE				
American Indian/ Alaskan Native	3	2	1	1
Asian	3	2	3	3
Native Hawaiian/Other Pacific Islander	0	0	0	0
Black/African American	2	2	6	6
White	99	89	92	90
Other/Hispanic	6	5	0	0
Total	**113**		**102**	
ETHNICITY				
Hispanic	15	13	20	20
Non-Hispanic	98	87	82	80
Total	**113**		**102**	

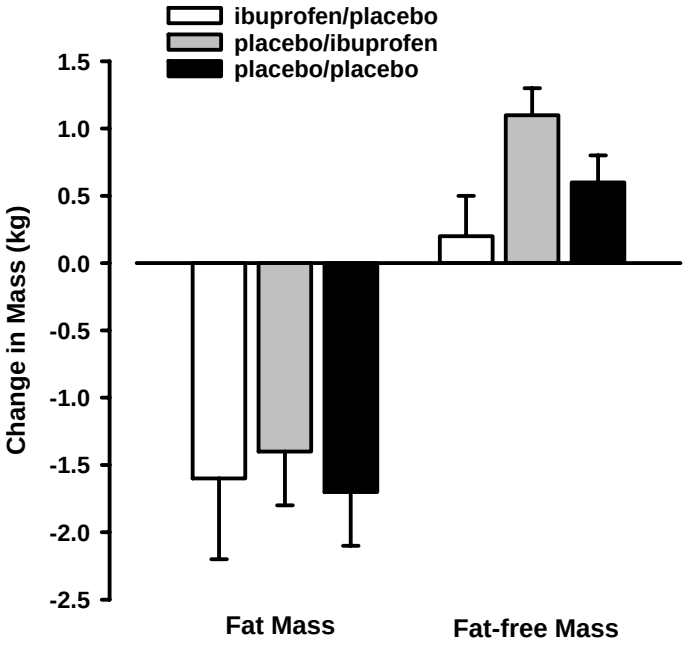

Figure 2. Changes in fat mass (kg) and fat-free mass (kg) in response to 9 months of exercise training.

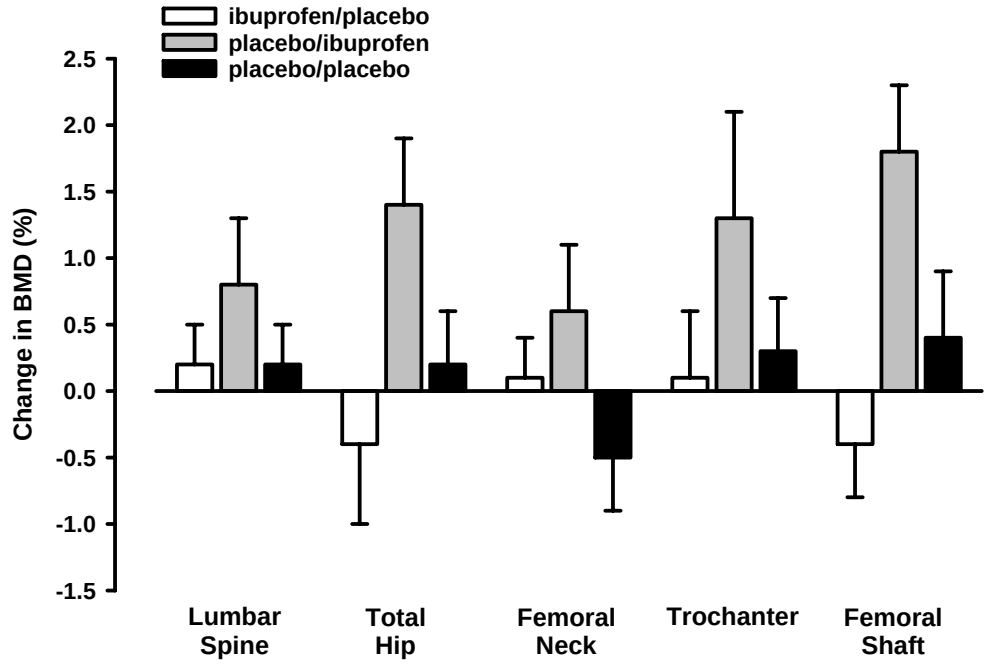

Figure 3. Changes in bone mineral density (%) in response to 9 months of exercise training.

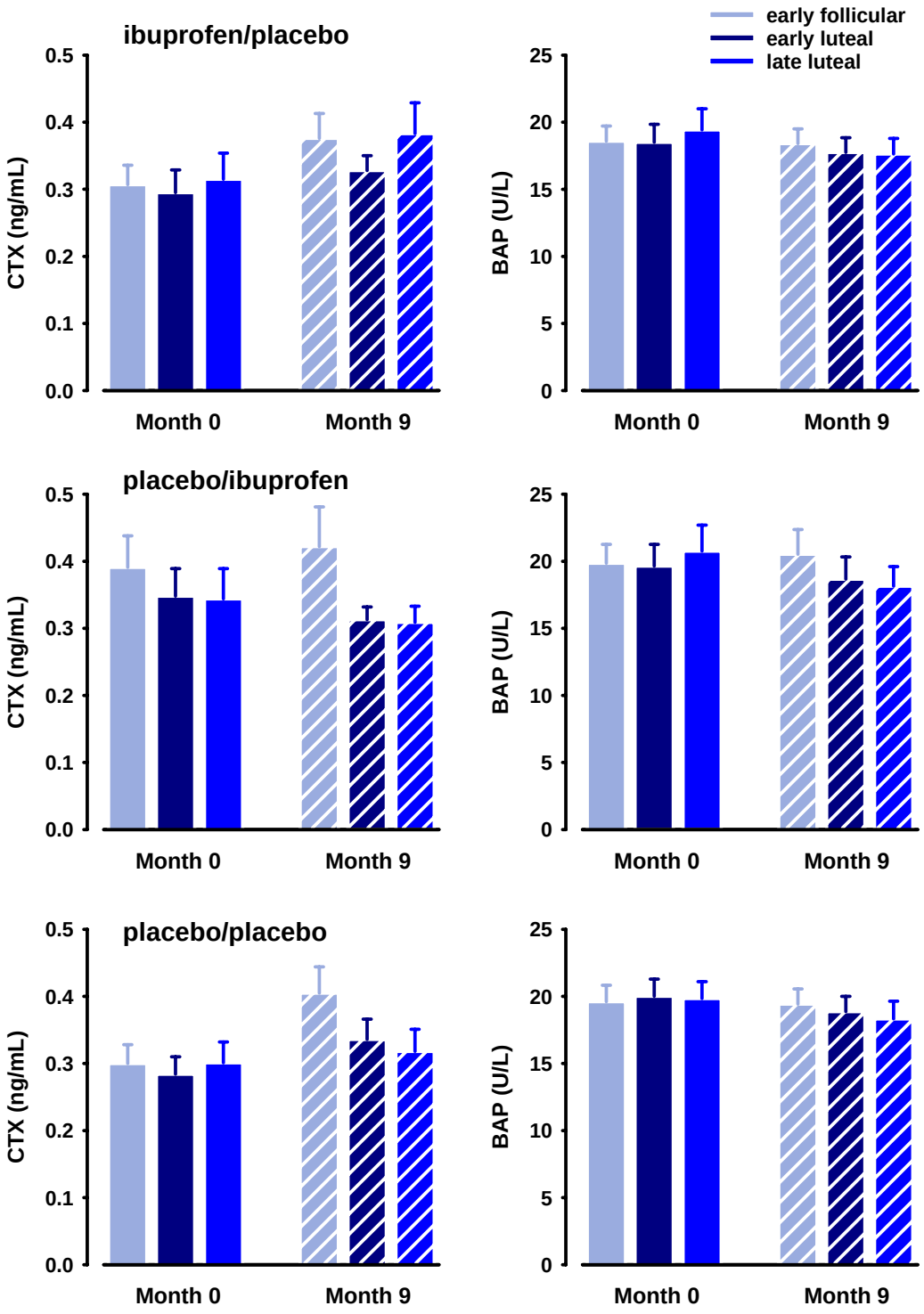

Figure 4. Markers of bone resorption (CTX) and formation (BAP) across the menstrual cycle before and after 9 months of exercise training.

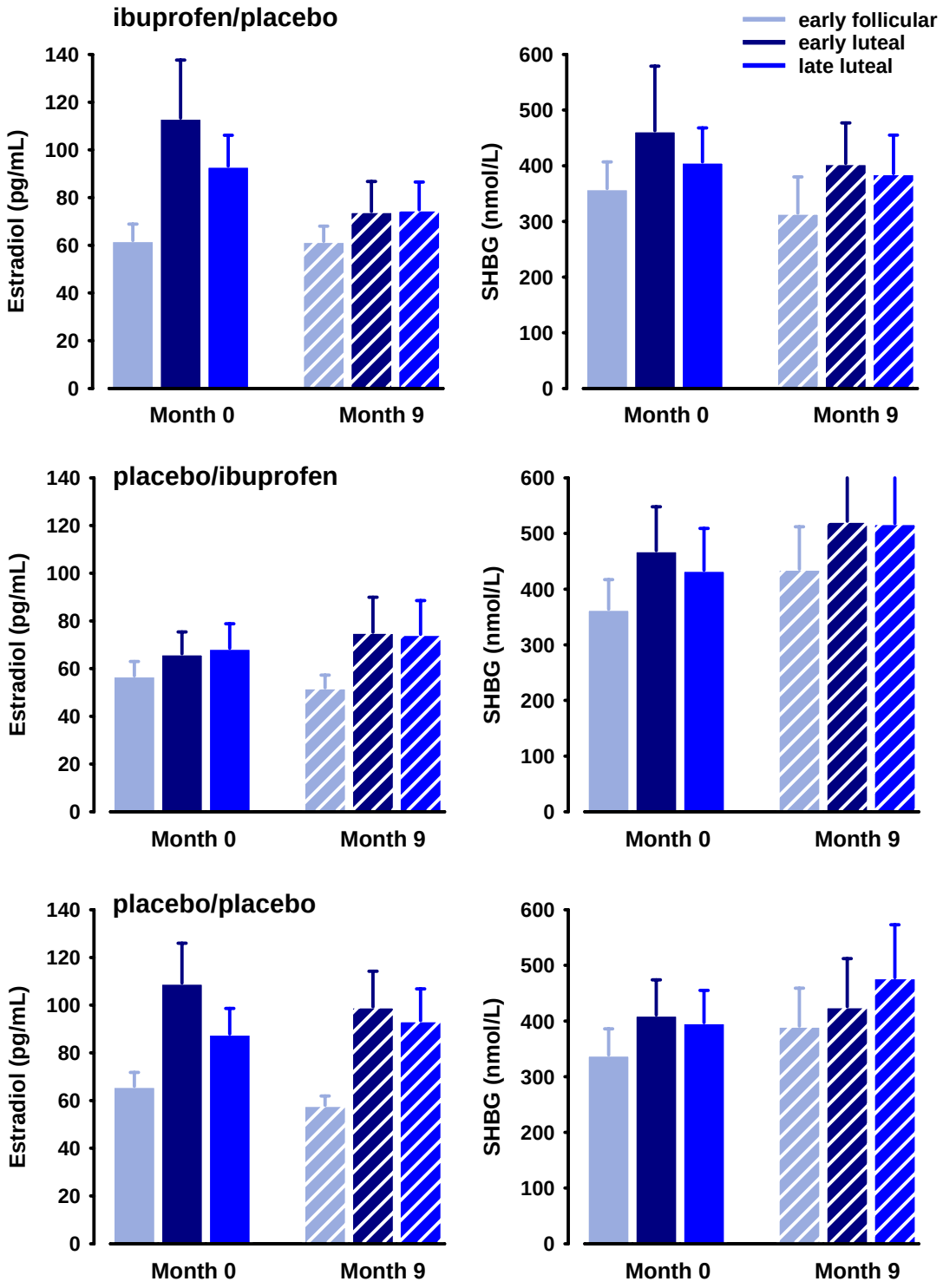

Figure 5. Serum estradiol and sex hormone binding globulin (SHBG) levels across the menstrual cycle before and after 9 months of exercise training.

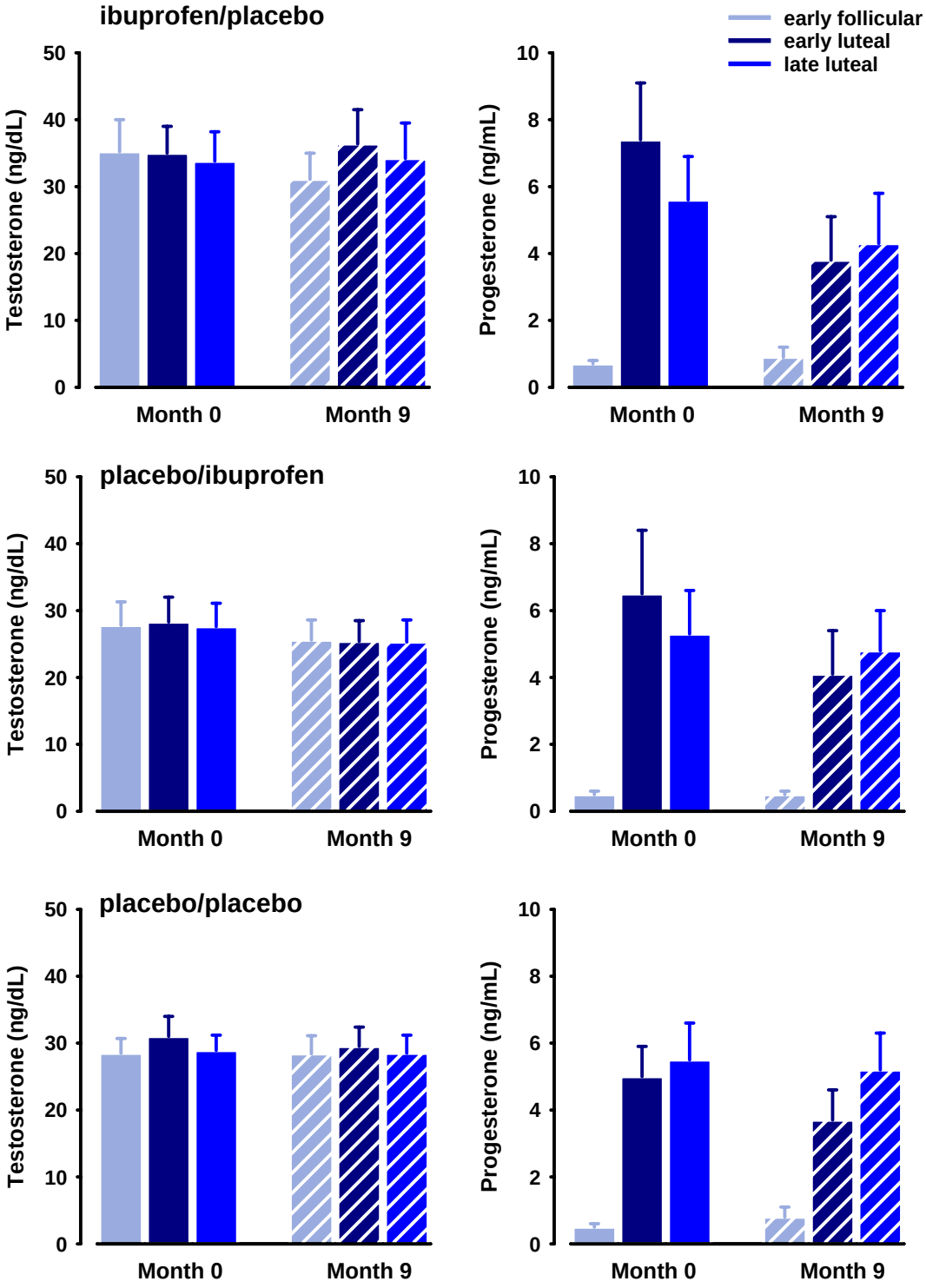

Figure 6. Serum testosterone and progesterone levels across the menstrual cycle before and after 9 months of exercise training.

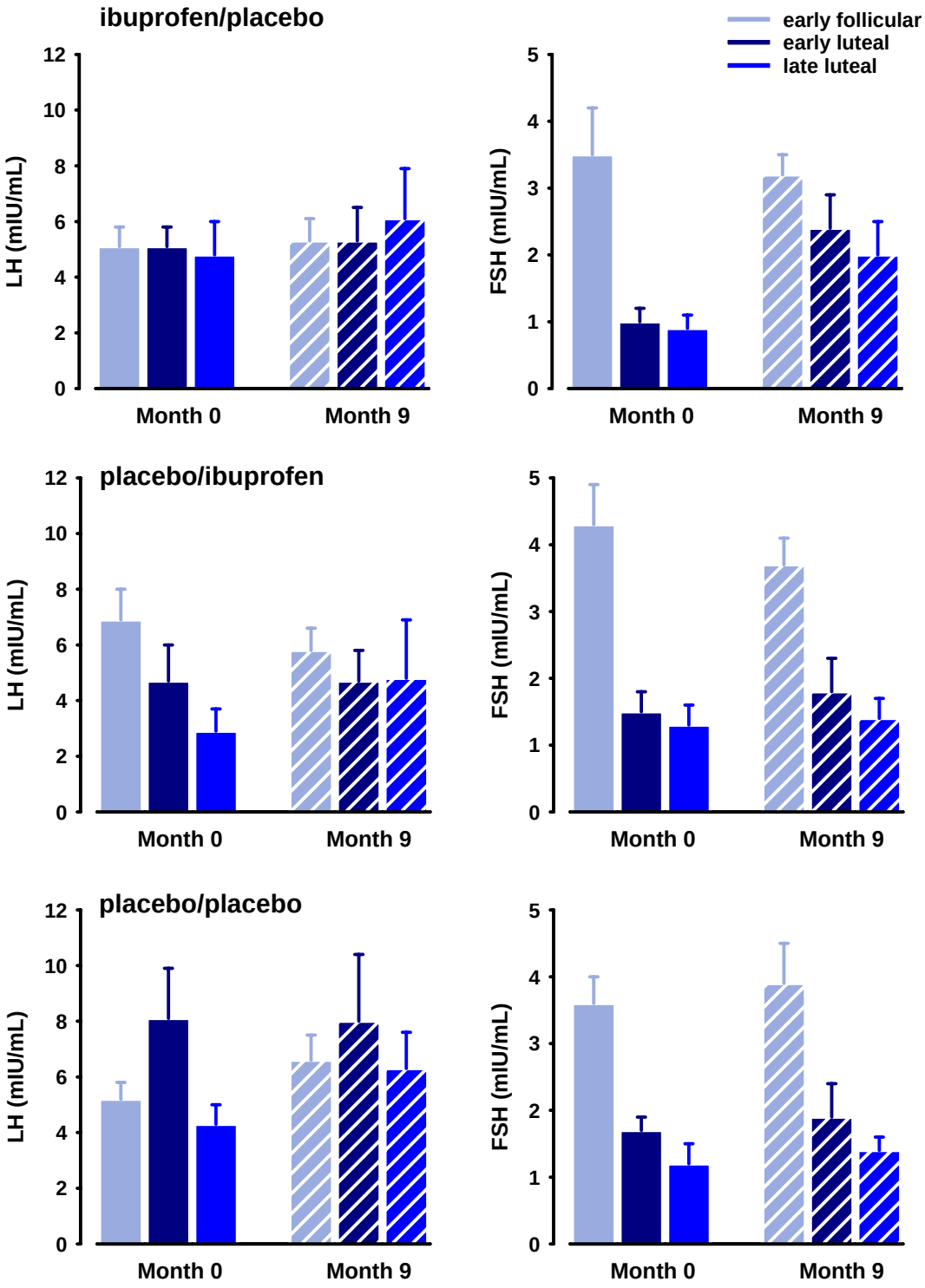

Figure 7. Serum luteinizing hormone and follicle stimulating hormone levels across the menstrual cycle before and after 9 months of exercise training.

Table 2. Dietary intake and cardiovascular fitness before and after 9 months of exercise training.

	ibuprofen/placebo		**placebo/ibuprofen**		**placebo/placebo**	
	before	**after**	**before**	**after**	**before**	**after**
Energy intake, kcal/d	1749±100	1635±397	1686±100	1825±68	1727±112	1759±106
protein, g/d	77±3	72±2	67±5	77±4	67±4	74±6
carbohydrate, g/d	202±15	192±15	224±19	221±7	218±14	217±14
fat, g/d	77±7	61±15	58±6	64±6	61±6	65±6
Calcium intake, g/d	897±141	790±95	1009±89	962±111	870±69	1058±124
VO_2max, mL/min/kg	33.7±0.8	37.3±1.1	33.4±1.2	36.4±1.4	31.3±0.9	35.1±1.0
HRmax, beats/min	190±2	186±2	188±2	185±2	189±2	187±2
RERmax	1.10±0.01	1.09±0.01	1.11±0.01	1.08±0.02	1.11±0.01	1.11±0.01
V_E/VO_2max	37.9±1.4	39.1±1.0	38.9±1.6	39.8±1.3	40.0±1.2	39.7±0.8

VO_2max=maximal aerobic power; HRmax=maximal heart rate; RERmax=maximal respiratory exchange ratio; V_E/VO_2max=maximal ventilatory equivalent; values are mean±SE